CHAPTER 1

INTRODUCTION

1.1 Flow injection analysis

Flow injection analysis (FIA) is a continuous flow technique which is ideally suited to rapid automated analysis of liquid samples. The technique was first described in Denmark by Ruzicka and Hansen in 1975[1], since this technique has been rapidly and widespread research, resulting in the publication of more than 15000 papers during few decade ago [2].

In a flow injection analyser, a small, fixed volume of a liquid sample is injected as a discrete zone via an injection device, into a liquid carrier which flows through a narrow bore tube or conduit. The sample zone is progressively dispersed into the carrier, initially by convection, and later by axial and radial diffusion, as it is transported along the conduit under laminar flow conditions. Reagents may be added at various confluence points and these mix with the sample zone under the influence of radial dispersion, to produce reactive or detectable species which can be sensed by any one of a variety of flow-though detection devices. The height or area of the peak-shaped signal thus obtained can be used to quantify the analyte after comparison with the peaks obtained for solutions containing known concentrations of the analyte.

Unlike batch methods which samples are held in individual vessels, in flow methods all sample are introduced at the same point and travel the same path with reproducible resident time. An example of a flow injection analyser arrangement

which consists of flow tubing, propulsion device, injection valve, mixing coils and flow through detector device are referred to collectively as a manifold. A simple two line manifold is shown in Figure 1.1, using the common symbolic notation. The bottom half of the diagram shows optionally-used devices for reagent and carrier propulsion, sample injection, sample-reagent mixing, and various detection modes.

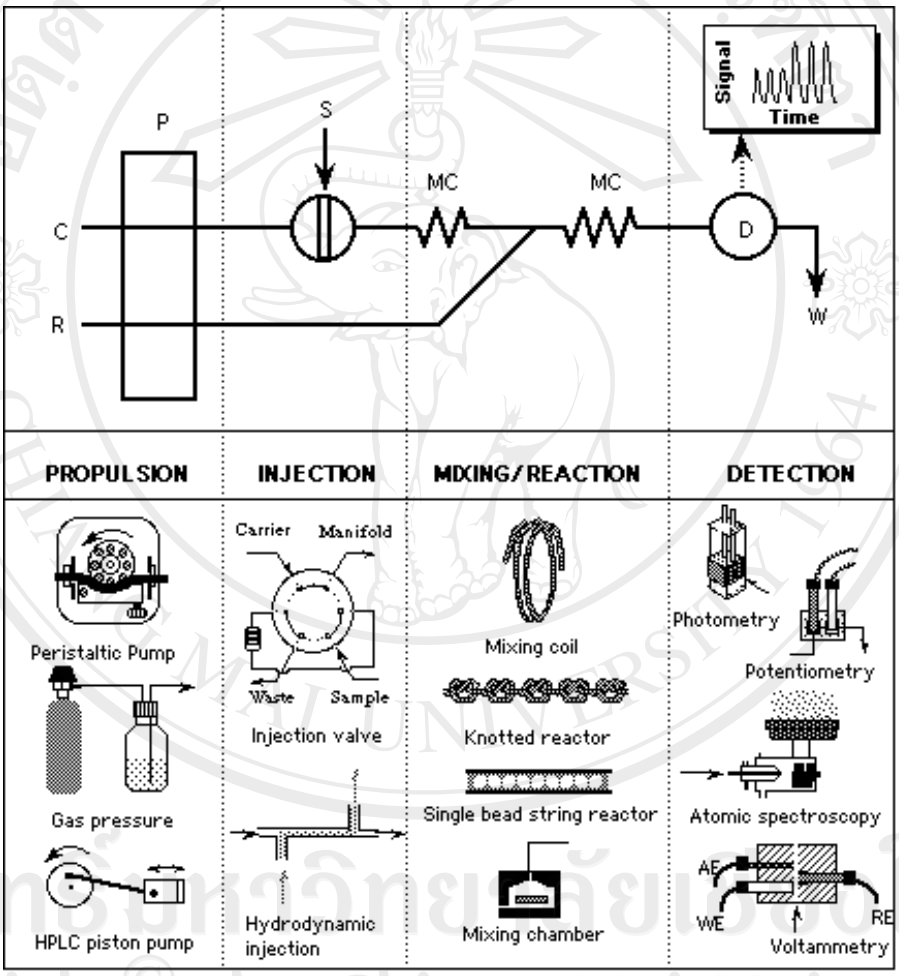

Figure 1.1 Schematic diagram of a typical flow injection analysis manifold (top). P is a pump, C and R are carrier and reagent lines respectively, S is sample injection, MC's are mixing coils, D is a flow through detector, and W is the waste container. The lower portion of the diagram indicates some typical instrumental options available for reagent and carrier propulsion, sample injection, sample-reagent mixing, and various detection modes [2].

Flow injection analysis is based on three principles which are critical for its successful operation and application. Firstly, reproducible timing, because the length of the manifold tubing remains constant and carrier and reagent flow rates vary little during transport of the sample zone from injection to the point of detection, the period for sample zone transport and mixing will be highly reproducible. In the second place, reproducible sample injection is achieved by the use of an injection valve with an injection loop of a defined volume. Thirdly, partial and controlled dispersion of the sample zone is critical for successful analysis - it provides the mechanism for mixing between sample and reagents, and prevents cross contamination between successive samples. The extent of this partial dispersion of the sample zone at the peak maximum is described by the dispersion coefficient, which is defined by:

$$D = C_0 / C = k_0 h_0 / k_1 h \qquad \ldots\ldots\ldots\ldots\ldots (1)$$

where D is the dispersion coefficient, C_0 is the concentration of the analyte in the undispersed sample, C is the concentration of the sample after it has been subject to dispersive processes, h_0 is the peak height for undispersed sample, h is the peak height for a dispersed sample. If signal is directly proportional to concentration, then $k_0 = k_1$, and D can be determined directly from the peak height ratio. Except when preconcentration is performed, the dispersion value must always be greater than unity.

Flow injection systems with $1<D<3$ are classified as having limited dispersion, and are used in conjunction with high sensitivity detection systems such as ion selective electrodes and atomic spectroscopy, where minimal sample dilution is necessary. Flow injection systems with $3<D<10$ are classed as medium dispersion, and are most commonly used in systems where significant sample and reagent mixing

is required, as is the case for methods involving spectrophotometric or fluorimetric detection. Large dispersion systems (D>10) are used where extensive mixing between sample and reagent is required, such as for flow injection titrations.

Dispersion can be controlled by manipulation of the sample volume and the hydrodynamic and geometric parameters of the manifold. For a single line flow injection system, the sample injection volume influences the magnitude of dispersion, according to:

$$1 / D = 1 - e^{-kSv} \qquad \text{..... (2)}$$

where k is a constant, and S_v is the sample injection volume. For uncoiled tubes, D has been shown to be proportional to the square root of L, the length of tubing travelled by the sample zone, and is approximately proportional to the internal diameter, d, of the flow tubing. However, for coiled or knotted tubes, the dispersion increase is minimal for an equivalent length of tubing. Flow rate may also influence the dispersion, but for a single line manifold, no major change in dispersion is observed over a flow rate range of 0.5 - 4 ml min^{-1} which is typical of that used in FIA.

1.1.1 Flow injection with electroanalytical measurements.

Flow injection analysis has been used in conjunction with a wide variety of electrochemical detectors (potentiometric, amperometric, conductimetric and

voltammetric) because feature a high sensitivity, wide linear range, selectivity against non–electrodes species, low cell dead volume and reduce costs [3].

Voltammetry comprises a group of electroanalytical techniques where information on the analyte is derived form current potential curves (IE curves). As a gradually increasing potential difference is applied between two electrodes, no current is theoretically produced – there is always some residual current, however, until the potential required for some analyte to be oxidized or reduced on the surface of working electrode is reached. At that time, the current rises abruptly to affixed value (the limiting current), which subsequently remains constant whatever the applied potential. In this way, a typical IE curve for the chemical species concerned is obtained. The study and application of IE curves to chemical systems have given rise to a variety of electroanalytical techniques. The most immediate classification of such techniques is based on whether the potential is scanned (voltammetry) or kept constant (amperomery). In the latter case, the analytical measured parameter is the relationship between the current and the analyte concentration. The many ways in which IE relations and available electrode types can be used has produced a host of electroanalytical techniques.

1.2 Fumaric acid

1.2.1 General background

Fumaric acid (trans – ethylene 1,2 dicarboxylic acid) is an organic acid molecules with molecular weight of 116.07 g/mol. Molecular structure of fumaric acid is shown in Figure 1.2.

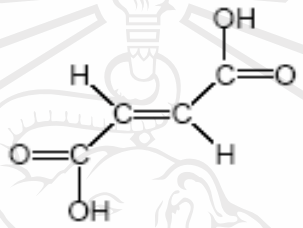

Figure 1.2 Molecular formula of fumaric acid.

In human body, fumaric acid acts as an intermediate of the tricarboxylic acid cycle (Figure 1.3). It is distributed in tissues of various species only at low concentration[4].

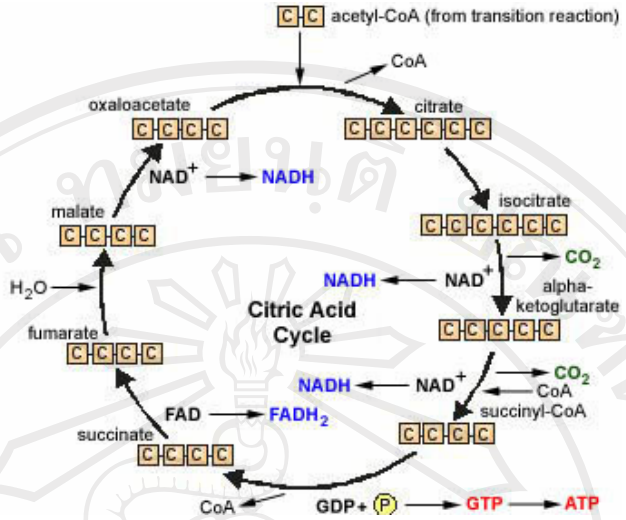

Figure 1.3 Tricarboxylic acid cycle[4].

Recently the commercial demand for fumaric acid has been increasing because of its extensive applications as a precursor in the synthesis of polymeric materials, an additive in paper and food industries.

Fumaric acid is added into fruit juice in order to prevent oxidation of ascorbic acid and to keep advantages of the drink. Fumaric acid provides more sourness per unit weight than other acidulants. The use of fumaric acid substantially reduces the acidulants cost in a fruit juice drink. In addition, it provides more buffering capacity than other acidulants when the pH is close to 3.0 as shown in Figure 1.4. It also helps to stabilize colour and flavor of food and drink.

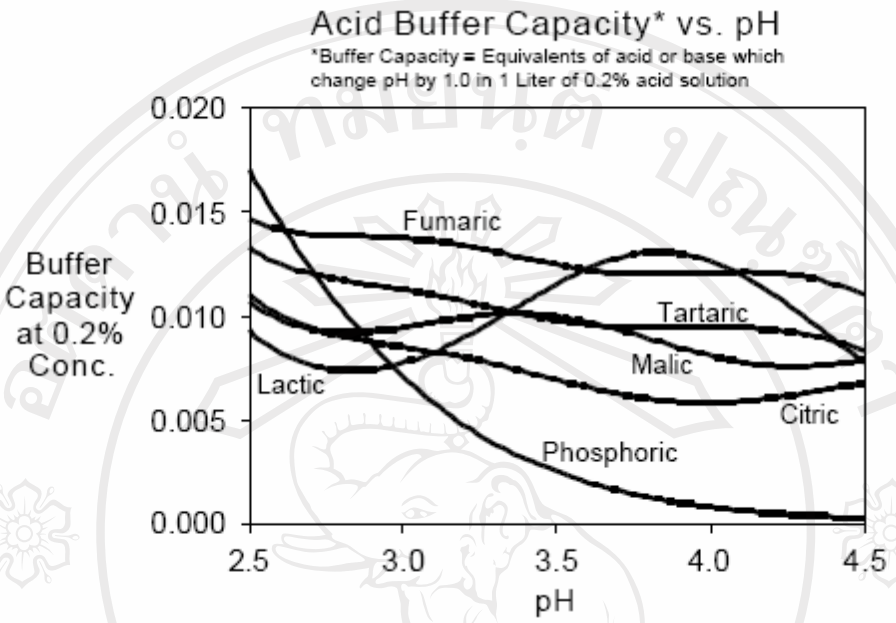

Figure 1.4 Buffer capacities of fumaric acid, tartaric acid, malic acid, lactic acid, citric acid and phosphoric acid buffer at different pH[5].

The hydrophobic nature of fumaric acid makes it an effective antimicrobial agent. Hydrophobic is important because the microbial cell wall normally contains lipid material and hydrophobic organic acid can interact with this lipid in a way that disrupts microbial activity. However, U.S. Food and Drug Administration (FDA) reports that fumaric acid additive must not exceed 25 part per million of the finish fruit juice drink [6], because ingestion of large amount may cause nausea, vomiting, cramps, and diarrhea and may produce irritation of the mouth and the throat.

1.2.2 Methods for fumaric acid determination

The standard method for the determination of fumaric acid is differential pulse voltammetry[7]. The method is based on the electrochemical reduction of fumaric acid on a hanging mercury drop working electrode to produce a measurable electrical current at potential of -1050 mV vs Ag/AgCl reference electrode.

High performance liquid chromatography (HPLC) is one of the popular technique [8]. Many methods based on different separation principles (ion exchange, ion – suppression and reversed – phase) and detection methods (UV absorption, conductimetric) have been developed. However, HPLC methods are very sensitive to matrix interferences and these interferences often precluded the reliable detection and/or accurate quantitation of some acids at level below 50 ppm[9]. Several methods are used for the determination of fumaric acid in real sample as summarized in Table 1.1.

Table 1.1 Methods for the determination of fumaric acid

Technique	Samples	Analyte	Linear range	r^2	Fumaric acid found	Ref. No.
HPLC (UV-Vis)	Honey	Organic acids	0.025 – 10.93 mg/kg	0.9998	0.07 – 5.15 mg/kg	[10]
Reversed-phase and ion – pair HPLC	Cucumber plants	Organic acids	0.1 – 0.2 µg/g	> 0.997	-	[11]
Ion-suppression reversed-phase HPLC	Pharmaceutical samples	Oxalic, fumaric, maleic and succinic acid	-	0.999	0.69 – 0.70 mg/L and 0.11 – 0.15 mg/L	[12]
HPLC	Rat plasma, urine, fecal	Fumaric acid	1 – 200 µg/mL 5 – 200 µg/mL 25 – 500 µg/g	-	-	[13]
HPLC	Fruit juice drinks	Organic acids and phenolic compounds	2 – 20 mg/L	0.9987	-	[14]
HPLC	Sediment	Organic acids	-	-	0.15 -13.1 µg/L	[15]
HPLC	Beer	Organic acids and phenolic acids	-	-	40 mg/l	[16]
Ion – exchange HPLC	Maple syrup	Organic acids	0.001 – 0.012 %	-	< 0.06 mg/L	[17]
Ion-exclusion LC	Fruit juices	Organic acids and sugars	5 – 100 mg/L	0.998	0.64 – 3.93 mg/L	[18]
IC (conductivity)	Atmospheric aerosol	Malic acid and other C_4 dicarboxylic acids	-	-	5.1 , 5.0 ng/m^3	[19]
IC (spectrophotometric detector)	Fruit juice	Fumaric acid, lactic acid, acetic acid	-	-	10 – 20 mg/L	[20]
Capillary electrophoresis (CE)	Chinese herb Sarcandar glabra and Medicinal preparation	Isofraxidin and fumaric acid	10 – 800 µg/mL	0.9992	647.2 µg/g 90.4 µg/mL 570.8 µg/tablet	[21]
cITP – CZE (UV)	Apple juice	Fumaric acid	25 –1000 ng/mL	0.9987	0.35 – 8.50 mg/l	[21]

Table 1.1 Methods for the determination of fumaric acid (continued)

Technique	Samples	Analyte	Linear range	r^2	Fumaric acid found	Ref. No.
2D Capillary isotachophoresis (conductivity and UV)	Feed additive	Organic acids, inorganic acids	0.05 - 2.5 mmol/L 0.02 – 0.12 mmol	0.999	42.9 %(W/W)	[23]
Ion-pair chromatography and capillary zone electrophoresis	Urine	Organic acids	0.1– 20.0 ppm and 0.7 –80 ppm	0.998 and 0.998	-	[4]
FIA spectrofluorimetry	Biological process	Fumaric acid	0.05 – 0.3 g/L 0.1 – 0.6 g/L	0.996 0.998	-	[24]
FIA (electrochemical detector)	coffee	acidity	$5.0x10^{-6}$ – $3.0x10^{-4}$ M		-	[25]
GC	Fruit juices	Organic acids	5 – 500 µg/mL	0.99	-	[26]
GC-MS	Distilled alcohol beverages	-	-	0.992 – 0.999	3ng/L	[27]
GC-MS	Box thorn leaves	Non-volatile organic acids	-	-	1.3 – 2.9 mg%	[28]
GC-MS-MS	Urine	Organic acids	-	1.000	-	[29]

1.3 Ascorbic acid

1.3.1 General background

Ascorbic acid is an organic acid and well known as vitamin C. Ascorbic acid appearance is white to light yellow crystals or powder and it is water soluble. It has an enediol configuration in the molecular structure thus its acidity derived from both hydroxyl groups on C_2 and C_3. The hydroxyl group on the C_3 carbon atom is more acidic than C_2 carbon atom with the dissociation constants are $pK_1 = 4.21$ and $pK_2 = 11.57$, respectively. The redox reaction potential of the first state of greatest

important biologically is E'_0 = +0.127 V. The molecular structure of ascorbic acid has an unusual flatness as shown in Figure 1.5.

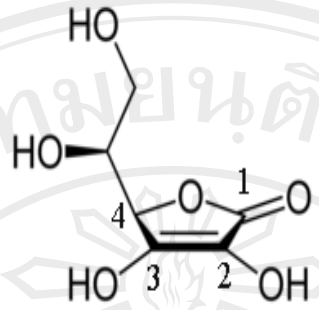

Figure 1.5 The molecular structure of ascorbic acid.

Ascorbic acid is an important micronutrient and plays many physiological roles in the body as a free radical scavenger, which may help to prevent free radical induced diseases such as cancer and Parkinson's disease. It is also vital to immune response, susceptibility of blood vessels to damage and bleeding, changes in bones and cartilage and retarded wound healing. It helps maintain elasticity of the skin, increase absorption of iron and improve resistance to infection. Moreover, ascorbic acid is necessary in redox processes taking place in cell that able to transport electrons and hydrogen. It is reversibly oxidized to dehydroascorbic acid (Figure 1.6) and partially metabolized to inactive sulfide and oxalic acid, which is expelled in urine. It is well absorbed from the digestive system and easily reaches the tissues.

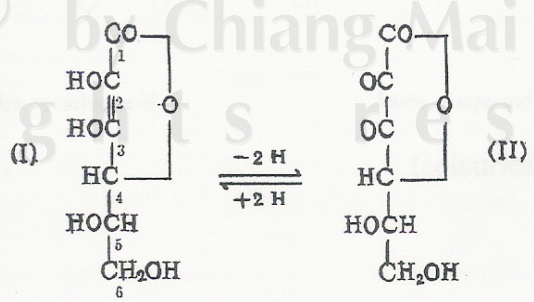

Figure 1.6 The reversible oxidation of ascorbic acid (I) to dehydroascorbic acid (II).

Ascorbic acid can not be synthesized by humans, but it is an essential dietary vitamin for the species (recommended daily intake of 60 mg) [30]. Ascorbic acid is one of the most common natural or artificially enriched ingredients in foods and beverages. However, the air oxidation of ascorbic acid in the presences of heat, light and iron makes the quantification of it in foods and beverages more important.

1.3.2 Methods for ascorbic acid determination

Owing to the wide use of ascorbic acid, many analytical techniques have been proposed for its determination in different matrices and at different concentration levels as summarized in Table 1.2. Moreover, due to the instability of ascorbic acid, the procedures on sample preparation for ascorbic acid determination are also summarized in Table 1.3.

Table 1.2 Methods for the determination of ascorbic acid

Technique	Samples	Analyte	Linear range	r^2/LOD	Ascorbic acid found	Ref. No.
Continuous-flow/stopped-flow with amperometric detector	Pharmaceutical formulations	Ascorbic acid	12 nM-3.5µM mg/kg	0.998/ 6nM	-	[31]
Differential pulse voltammetry with modified electrode	-	Ascorbic acids	$1x10^{-4}$-$1x10^{-5}$M	-/sub µM	-	[32]
Circulatory flow injection	Pharmaceutical products	Ascorbic acid	5-25 mg/L	-/-	105-984 mg/g	[33]
Capillary zone electrophoresis with UV spectrophotometry	Fruit juices	Ascorbic acid and sorbic acid	2.45-352.00 mg/L	0.9969/ 1.70 mg/L	53-898 mg/L	[34]
Spectrofluorimetry	Pharmaceutical, fruits, vegetables and soft drinks	Ascorbic acid	0.02 – 0.36 µg/mL	-/0.012 µg/mL	-	[35]
Spectrophotometry	Beverages and pharmaceuticals	Ascorbic acids	$8.0x10^{-6}$-$8.0x10^{-5}$ M	0.999 / -	-	[36]
Electroanalysis with modified electrode	-	Ascorbic acid, dopamine and uric acid	4.0-10 µM	0.9958 / -	-	[37]

Table 1.2 Methods for the determination of ascorbic acid (continued)

Technique	Samples	Analyte	Linear range	r^2/ LOD	Ascorbic acid found	Ref. No.
Electroanalysis with modified electrode	-	Ascorbic acid and uric acid	2.0-130 µM	0.9999 / -	-	[38]
Square wave voltammetry	Human urine	Uric acid and ascorbic acid	1.0-8.0 µM	0.9985 / -	3.84-4.26 µM	[39]
Electrochemicall-controllable chemiluminescence with modified electrode	-	Ascorbic acid and glucose	0.02 – 0.1 mM	-	-	[40]
Spectrofluorimetry	Vitamin C tablets, orange juice and nutrimental water drink	Ascorbic acids	0.05-8.0 µg/mL	0.9991/ 0.012 µg/mL	-	[41]
HPLC	Human milk	Ascorbic acid	0.5-100 µg/mL	0.999/-	-	[42]
LC using polymeric column with UV detector	Wines	Ascorbic acid	1-200 mg/L	0.999/ -	23-110 mg/L	[43]
Cyclic voltammetry with modified electrode	-	Ascorbic acid, epinephrine and uric acid	$2.0x10^{-5}$-$1.0x10^{-3}$ mol/L	-/ $7.0x10^{-6}$ mol/L	-	[44]
Electroanalysis with modified electrode	Fruits	Ascorbic acids	$4.0x10^{-8}$-$1.0x10^{-4}$ mol/L	- / $1.5x10^{-8}$ mol/L	3.42-58.88 mg/100g	[45]
HPLC-UV	Fortified food products	Ascorbic acid	1-100 µg/mL	0.9999 / -	-	[46]
HPLC-ESI-MS	Multivitamin tablets	10-water soluble vitamins	0.02-50 mg/L	0.9963 / 12 µL	-	[47]
Muti-pumping flow system with chemiluminometry	Powdered materials for preparation of fruit juice	Ascorbic acid	0.8-11.5 mmol/L	0.9984 / 0.17 mmol/L	25-43 mg AA/100 mL	[48]
Capillary zone electrophoresis with electrochemical detector	Grape fruit peels and juices	Ascorbic acid	$5x10^{-6}$-$1x10^{-4}$ g/mL	0.9978 / $1.0x10^{-6}$ g/mL	-	[49]
Spectrophotometric method using methyl viologen reagent	Pharmaceutical products, fruit juices and vegetables	Ascorbic acid	1.0-10 µg/mL	- / 0.1 µg/mL	-	[50]
Spectrophotometry using CUPRAC reagent	Grape juices	Ascorbic acid	$(0.5\text{-}3)x10^{-5}$ M	0.999 / -	-	[51]
Capillary zone electrophoresis with amperometric detector	Black tea	Sugar and ascorbic acid	$1.0x10^{-6}$-$5.0x10^{-5}$ mol/L	0.9979 / $1x10^{-7}$ mol/L	-	[52]

Table 1.3 Samples preparation for ascorbic acid determination

Sample	Sample preparation	Detection	Ref. No.
Pharmaceutical and food	- Potassium phosphate at pH 6.0 and 7.0 used as an electrolyte	FI - Amperometry	[53]
Pharmaceutical and food	- 0.05 M phosphate buffer in 0.05 M KCl, pH 6.5 used as carrier. - Juice and sport drink samples were directly diluted in working buffer	FI - Amperometry	[54]
-	- Phosphate buffer solution (0.2M, pH 7) used as an electrolyte	Voltammetry	[55]
-	- 0.1 M phosphate buffer adjusting pH by using sodium hydroxide that used as an electrolyte	Cyclic Voltammetry and amperometry	[56]
Pharmaceutical formulations	- Unstable solutions (ascorbic acid and the samples) were freshly prepared just before use. - Acetic acid and sodium acetate are used to prepare the buffered electrolyte	FI - Amperometry	[57]
Urine	- All sample solutions were diluted with a monochloroacetic acid solution previously purge with nitrogen	FI - Amperometry	[58]

Table1.3 Samples preparation for ascorbic acid determination (continue)

Sample	Sample preparation	Detection	Ref.
Biological matters	- Dissolving the sample in distilled water - The solution was prepared fresh every day and kept in the dark and cold place to minimize oxidation	FI - Spectrophotometry	[59]
Pharmaceutical products	- Ascorbic acid was prepared immediately before use by dilution stock solution in a 0.014 M nitric acid medium with deaerated water	FI - Spectrophotometry	[60]
Vitamin and soft drinks	- The sample was injected directly into the acetate – buffer – flow tube	UV/Vis detector	[61]
Pharmaceutical products	- Dissolving ascorbic acid in 0.01 M perchloric acid - Working solution was diluted with ultra pure water - All solutions were kept in amber colored bottles in the dark.	Fluorescence detection	[62]
Beverages	- The samples were filtrated and diluted with 50% ethanol solution	Electron spin resonance	[63]

1.4 Thesis aims

The aims of this research work are summarized as follows:

1. To develop a simple flow injection system with differential pulse voltammetric system for the preliminary determination of fumaric acid
2. To develop flow injection – amperometric method with dialysis sample pretreatment for determination of ascorbic acid in fruit juice and vitamin C tablet samples